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GB Virus C Prevalence in Blood Donors and High Risk Groups for Parenterally Transmitted Agents From Gauteng, South Africa†

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The prevalence of GBV-C infection in voluntary blood donors and in groups at high risk for parenteral exposure to infectious agents was studied. The high risk groups included chronic renal failure patients on haemodialysis, renal transplant patients and haemophiliacs from Gauteng. The presence of GBV-C RNA in these populations was determined using reverse transcription polymerase chain reaction (RT-PCR) in the 5′ non-coding region (NCR) of the virus. Of the blood donors, 11.1% (95% CI 7.6, 15.8) were positive, whereas 23.8% (95% CI 12.6, 40.2) of haemodialysis patients and 23.5% (95%CI 15.9, 33.3) of the haemophiliacs were infected with GBV-C. The highest proportion of infection was in the renal transplant patients, where 41.2% (95% CI 35.1, 47.7) were found to have circulating GBV-C RNA. Serological markers for hepatitis B (HBV) and hepatitis C viruses (HCV) were also measured as indicators of other hepatitis viruses with important parenteral transmission routes. Of the GBV-C positive blood donors, 3.6% were also HBsAg positive and none were positive for HCV. The GBV-C positive patients on haemodialysis were not positive for either HBsAg or antibodies to HCV, but had evidence of past infection with HBV since 40% were anti-HBc positive. The greatest proportion of HCV positives was in the haemophiliac group, 91.3%, none of these were HBsAg positive but 39.1% had anti-HBc. In the GBV-C positive renal transplant patients, 4% had HBsAg, 13.3% had anti-HBc and 2.1% had antibodies to HCV. This is the first report describing the prevalence of GBV-C in South African populations. ***J. Med. Virol. 55: 103–108, 1998.***

KEY WORDS: GBV-C; epidemiology; parenteral transmission

INTRODUCTION

Viral hepatitis is known to be caused by a number of viral agents, designated hepatitis A, B, C, D and E. Sensitive and specific tests are available for the detection of all of these viruses, yet the etiology of 10 to 20% of post-transfusion and community-acquired non-A-E hepatitis has remained unclear, suggesting that additional viruses may be implicated [Alter, 1996]. Two possible candidates have recently been identified, designated GB virus C [Simons et al., 1995] and hepatitis G virus [Linnen et al., 1996]. Both are single-stranded RNA viruses belonging to the Flaviviridae family and show nucleotide sequence identities of approximately 85% [Schlueter et al., 1996], indicating that they are independent isolates of the same virus [Zuckerman, 1996]. In this report we refer to this virus as GB virus C (GBV-C).

There is little information about the epidemiology, mode of transmission and pathology associated with GBV-C, and conflicting reports regarding association with disease have been published. The virus has been detected in patients with fulminant hepatitis [Yoshiba et al., 1995; Mishiro et al., 1996], although its link with severe liver damage has been questioned [Kuroki et al., 1996; Kao et al., 1996]. Other studies have suggested that infection with GBV-C is not associated with recur-

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†Informed consent has been obtained from patients or their parents/guardians for patients less than 18 years of age. Clearance from the Committee for Research on Human Subjects and Ethics at the University of the Witwatersrand, Johannesburg has been obtained, protocol number M970203, and human experimentation guidelines as specified by this committee were followed in the conduct of the clinical research.

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TABLE I. PCR Primers for Amplification of the 5′ NCR of GBV-C RNA

Primer	Sequence	Position*
Outer sense	5′-GCCAAAAGGTGGTGGATGGG-3′	102
Outer antisense	5′-ACTGGTCCTTGTCAACTCGCCG-3′	376
Inner sense	5′-TGATGACAGGGTTGGTAGGTCG-3′	122
Inner antisense	5′-GGTCAAGAGAGACATTGAAAGGG-3′	342

*The nucleotide position refers to the position on U44402 on Genbank.

rent or severe liver disease [Belli et al., 1996], although an association between GBV-C infection and aplastic anaemia has been suggested [Byrnes et al., 1996; Zaidi et al., 1996].

Initial reports indicate varying prevalences of GBV-C infection in blood donor populations from around the world, ranging from 0.9% in Japan [Masuko et al., 1996] to 12.9% in a group of commercial blood donors from the USA [Dawson et al., 1996], with intermediate figures from volunteer donors in Australia [Moaven et al., 1996]. GBV-C has also been reported in various high risk groups associated with parenteral transmission of infectious agents, specifically blood and blood product recipients [Neilson et al., 1996; Schmidt et al., 1996], patients on maintenance haemodialysis in Indonesia [Tsuda et al., 1996], Japan [Masuko et al., 1996] and France [De Lamballerie et al., 1996], leprosy patients [Egawa et al., 1996] and intravenous drug users [Aikawa et al, 1996]. Further modes of transmission of the virus have been proposed, including vertical transmission [Lin et al., 1996; Feucht et al., 1996; Moaven et al., 1996] and sexual transmission [Stark et al., 1996; Rubio et al., 1996].

We describe the epidemiology of GBV-C in groups at high risk for parenterally transmitted viruses and in blood donors from the province of Gauteng, in South Africa. The high risk groups chosen for investigation were chronic renal failure patients on haemodialysis, renal transplant patients and patients from the haemophiliac clinics in Gauteng. Volunteer blood donors were chosen from the same province, to give an indication of the background population positivity for GBV-C. Since at present there is no reliable serological screening assay for GBV-C, population surveys are limited to detection of the viral genome. This is the first report from South Africa that describes the prevalence and epidemiology of GBV-C infection in this country using RT-PCR techniques to detect GBV-C RNA in blood donors and groups at high risk for parenteral exposure.

MATERIALS AND METHODS

Samples

Five hundred and thirty-two specimens from blood donors were obtained from the South African Blood Transfusion Service in Gauteng. The patients were stratified by race and included regular donors (n = 316) and also first-time blood donors (n = 197). For comparison, sera from individuals at high risk for parenteral infections was obtained from haemophiliacs (n = 102), chronic renal failure patients on haemodialysis (n = 42) and renal transplant patients (n = 245) at the Johannesburg General hospital in Gauteng. These patients represented the entire cohort of patients attending the relevant specialist clinic at the time of the study. All sera were stored at −70°C until processing.

Biochemicals

Reagents used for the reverse transcription (RT) and the polymerase chain reaction (PCR) were from Boehringer Mannheim (Germany). RNA was extracted using the QIAamp Viral RNA Kit (Qiagen, Germany). The Hepatitis G Virus Primer and Capture Probe Set was from Boehringer Mannheim. Sequencing was done directly on PCR products using the Sequenase PCR Product Sequencing Kit (United States Biochemicals).

Oligonucleotides

Oligonucleotides (Table I) were designed based on alignment of the 5′ non-coding regions of GBV-C (Genbank Accession Number U36380) and hepatitis G virus (Genbank Accession Numbers U44402 and U45966). The sizes of the first and second round PCR products were 296 and 242 base pairs respectively.

RT-PCR

GBV-C RNA was isolated using the QIAamp Viral RNA Kit according to the manufacturer's instructions. A combined RT-PCR was carried out in 0.5 μl thin-walled tubes in a 50 μl reaction volume. The RT-PCR mixture contained final buffer concentrations of 10 mM Tris-HCl, pH 8.6, 50 mM KCl, 2 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100 (as modified from Chen et al., 1992). $(NH_4)_2SO_4$ was added to a final concentration of 15 mM. The other reaction components were 0.2 mM of each dNTP, 2.5 U each of Taq DNA Polymerase, AMV Reverse Transcriptase and RNAsin, and 20 pmol of each outer primer. Reverse transcription was at 43°C for 60 min, followed immediately by 35 cycles of 94°C for 80 sec, 55°C for 60 sec, and 72°C for 80 sec. The final amplification cycle was followed by a 10 min extension at 72°C. The second round of PCR was carried out with the inner primers, using the PCR buffer of Chen et al [1992]. Cycling conditions for the second round were 94°C for 80 sec, 50°C for 60 sec, and 72°C for 80 sec, again for 35 cycles. A 10 min extension at 72°C was again carried out. Controls in each case were water, and a negative control serum (nonreactive on multiple assays and derived from a laboratory worker) which had been extracted in parallel with the test specimens.

To confirm the positive results in the blood donor population, the Boehringer Mannheim Hepatitis G Vi-

TABLE II. Prevalence of GBV-C in Study Groups

	Males				Females				Total			
Group	n	+ve	%	95% CI	n	+ve	%	95% CI	n	+ve	%	95% CI
Blood donors	275	39	14.2%	[8.9, 21.9]	257	20	7.8%	[4.0, 14.4]	532	59	11.1%	[7.6, 15.8]
Haemodialysis	15	6	40.0%	[17.5, 69.0]	27	4	14.8%	[4.9, 35.1]	42	10	23.8%	[12.6, 40.2]
Renal transplant	143	62	43.4%	[35.2, 51.9]	102	39	38.2%	[28.9, 48.6]	245	101	41.2%	[35.1, 47.7]
Haemophiliac	97	23	23.7%	[15.9, 33.7]	5	1	20.0%	[1.1, 73.8]	102	24	23.5%	[15.9, 33.3]

rus Primer and Capture Probe Set was used according to the manufacturer's instructions. A total of 49 GBV-C positives were repeated using this kit.

Detection of products

PCR products were analysed either by electrophoresis on a 2% agarose gel and visualised by UV fluorescence after staining with ethidium bromide, or for the commercial HGV Kit, a plate detection system was utilised according to the manufacturer's instructions.

Sequencing of PCR products

The specificity of the amplified products was assessed by direct sequencing using a commercial sequencing kit.

Serological Markers

Serum samples were tested for anti-HCV with a third generation enzyme linked immunosorbent assay (Murex, Anti-HCV III). Serum samples were tested for hepatitis B virus (HBV) surface antigen (HBsAg), antibody to HBV surface antigen (anti-HBs) and for antibodies against HBV core antigen (anti-HBc) using radioimmunoassay (Abbott, AUSRIA, AUSAB). HIV testing was performed with the Murex HIV 1+2 kit and TPHA with the Murex Welcosyph HA kit following the manufacturer's instructions.

Statistical Analysis

The proportion of GBV-C positives was calculated for each group. Data on the racial breakdown of Gauteng blood donors were used to weight the results for each stratum of the blood donor sample. These were then combined to estimate the GBV-C positivity in the general blood donor population of Gauteng. Confidence intervals of 95% were calculated using the method of Fleiss (1981). Comparisons between groups were done by a Mantel-Haenszel χ^2 test adjusted for age [1959]. A P value less than 0.05 was considered significant.

RESULTS

Table II shows the prevalence of GBV-C in each group differentiated by sex. Although the males showed higher levels of GBV-C positivity, the differences between males and females were not statistically significant for any of the groups ($P > 0.05$). After weighting, the prevalence of GBV-C in blood donors was estimated at 11.1% whereas 23.8% of the haemodialysis patients, 41.2% of the renal transplant patients and 23.5% of the haemophiliac patients were positive for GBV-C. Each of these risk groups had statistically significantly higher GBV-C positivity than blood donors (Mantel-Haenszel χ^2 test adjusted for age: haemodialysis $\chi^2_{MH} = 4.7$, $P = 0.03$; renal transplant $\chi^2_{MH} = 56.9$, $P = 0.001$; haemophiliac $\chi^2_{MH} = 6.9$, $P = 0.009$). When analysed separately, the GBV-C positivity in black donors was 29.0% (95% CI 23.9, 34.8) compared to 8.5% (95% CI 5.5, 12.9) in white blood donors. This difference was statistically significant ($\chi^2 = 35.3$, $P = 0.001$). There was no statistically significant difference between the first time and regular blood donors with regard to GBV-C positivity, with 13% and 10% positive respectively ($\chi^2 = 3.15$, $P = 0.076$).

Sequencing was carried out directly on PCR products, and the sequences generated were compared with those available for GBV-C. A total of 48 5′ NCR sequences were obtained, which, when aligned with known GBV-C sequences on Genbank, showed homologies from 83% to 98% (results not shown). With the commercial Hepatitis G Virus Primer Capture and Probe Set, 49 of the specimens that were positive by the 5′ NCR PCR were re-extracted and assayed. Of these, 44 (89.8%) were positive with the commercial assay.

The association between GBV-C positivity and the presence of various markers of HBV and HCV infection is shown in Table III and Table IV. HIV and TPHA results were available for the blood donors since they are routinely measured, and are shown in Table III. Of the 510 blood donors tested for HBsAg, 6 were positive (1.2%), and 4 out of 511 (0.8%) had antibodies to HCV. HIV and TPHA markers were found at rates of 1.0% and 1.6% respectively. None of the 42 haemodialysis patients were positive for HBsAg or had antibodies to HCV, but 31.0% had anti-HBs and 31.0% had anti-HBc. 6 of 241 renal transplant patients tested had HBsAg (2.5%), 58 out of 240 (24.2%) had anti-HBs, 38 out of 238 (16%) had anti-HBc, and 14 out of 234 (6%) had anti-HCV. The presence of hepatitis markers was prevalent particularly in the haemophiliac population: anti-HCV was present in 83.0%; 1.0% had HBsAg; 78.6% had anti-HBs; and 41.4% had anti-HBc.

Table IV shows co-infection of HBV and HCV in patients found positive for GBV-C. HBsAg was present in 4% of GBV-C positive renal transplant patients and 3.6% of the blood donor group, indicating chronic infection with HBV. Anti-HBc was high in both haemodialysis patients (40%) and in the haemophiliac group (39.1%). The co-infection rate in the haemophiliac group was very high at 91.3% for HCV. In the other groups tested there was no marked association of GBV-C infection and other viral hepatitis markers.

TABLE III. Comparison of GBV-C and HBV and HCV and Other Infectious Markers in Blood Donors and Risk Groups

Group	GBV-C		HBsAg		Anti-HBs		Anti-HBc		Anti-HCV		HIV		TPHA	
	n	+ve (%)	n	+ve (%)	n	+ve (%)	n	+ve (%)	n	+ve (%)	n	+ve (%)	n	+ve (%)
Blood donors	532	57 (10.7%)	510	6 (1.2%)		ND		ND	511	4 (0.8%)	510	5 (1.0%)	512	8 (1.6%)
Haemodialysis	42	10 (23.8%)	42	0 (0.0%)	42	13 (31.0%)	42	13 (31.0%)	42	0 (0.0%)		ND		ND
Renal transplant	245	101 (41.2%)	241	6 (2.5%)	240	58 (24.2%)	238	38 (16.0%)	234	14 (6.0%)		ND		ND
Haemophiliac	102	24 (23.5%)	99	1 (1.0%)	98	77 (78.6%)	99	41 (41.4%)	100	83 (83.0%)		ND		ND

ND: Not done.

DISCUSSION

This is the first study determining the prevalence of GBV-C in South Africa. The study was carried out in the province of Gauteng which has the highest population density in the country.

The prevalence of 23.8% of GBV-C in the haemodialysis patients is in line with the prevalences reported for haemodialysis patients elsewhere. Studies have shown results ranging from 3.1% [Masuko et al., 1996] for haemodialysis patients in Japan, to as high as 57.5% [De Lamballerie et al., 1996] in a study in France. A similar figure was found in the haemophiliac population that we studied, with lower prevalences reported for haemophiliacs in Scotland (14%) [Jarvis et al., 1996] and in Europe (18.4%) [Linnen et al., 1995]. The renal transplant patients had the highest frequency of GBV-C viraemia at 41.2%. This is not unexpectedly high, since these patients receive immunosuppressive therapy, and it has been documented that immunosuppression may increase the risk of GBV-C infection [Neilson et al., 1996].

The prevalence of GBV-C in haemophiliac populations is relatively low when the multi-transfused nature of their condition is considered. Examining the prevalence of other viral markers in the group of haemophiliacs, it is clear that HCV is present at a much higher frequency than GBV-C, with frequencies of 83% and 23.5% respectively. Jarvis et al. [1996] have given two possible explanations for this phenomenon. The first is that GBV-C RNA is cleared by the immune system, however, several groups [Nakatsuji et al., 1996; Leary et al., 1996; Dawson et al., 1996] have noted the existence of persistent viraemia, with GBV-C RNA positivity for up to 10 years in one study [Masuko et al., 1996]. This anomaly is yet to be resolved, but the explanation of persistent viraemia would help to explain the high prevalence in the blood donor population. The second possibility for the difference in frequency between HCV and GBV-C prevalence in the haemophiliac group is that GBV-C could have a lower rate of infectivity than HCV or be more readily inactivated [Jarvis et al., 1996]. Solvent detergent inactivation of blood products has been shown to completely eliminate GBV-C RNA, but heat treatment of blood products was only able to reduce the level of GBV-C, without complete elimination [Jarvis et al., 1996].

The only remarkable co-infection finding was the association between GBV-C and HCV infection in the haemophiliac group. The very high level of HCV infection in this group is probably indicative of the fact that they were exposed to blood or blood products unscreened for HCV. This could be clarified in future studies of age-stratified haemophiliac cohorts. However, previous exposure to hepatitis B virus as indicated by the presence of anti-HBc is substantial in both the haemophiliacs and the haemodialysis patients.

Chronic carriage of HBV does not seem to be associated with GBV-C positivity, since the numbers of patients with HBsAg in the GBV-C positive groups are

TABLE IV. Presence of HBV and HCV Markers in Patients Positive for GBV-C in Risk Groups

	Total	HBsAg			Anti-HBs			Anti-HBc			Anti-HCV		
Group	GBV-C $^{+}$ve	n	$^{+}$ve	%	n	$^{+}$ve	%	n	$^{+}$ve	%	n	$^{+}$ve	%
Blood donors	59	55	2	3.6%		ND			ND		55	0	0.0%
Haemodialysis	10	10	0	0.0%	10	3	30.0%	10	4	40.0%	10	0	0.0%
Renal transplant	101	99	4	4.0%	99	25	25.3%	98	13	13.3%	94	2	2.1%
Haemophiliac	24	23	0	0.0%	23	19	82.6%	23	9	39.1%	23	21	91.3%

low in all groups studied. These results are comparable to a previous publication [Schlueter et al., 1996].

A noteworthy finding in this study was the prevalence of GBV-C in blood donors in Gauteng. This was found to be much higher, at 11.1%, than figures reported for other parts of the world, with the exception of West Africa at 14.2% [Dawson et al., 1996] and 12.9% in a commercial blood donor population from the USA [Dawson et al., 1996]. The next highest reported figure is from Germany at 4.7% [Herringlake et al., 1996].

To validate the high prevalence of GBV-C in the blood donors, the commercial assay for hepatitis G RNA from Boehringer Mannheim was used on a sample of positives. This showed that 89.8% of the specimens positive for GBV-C RNA with our own assay were also positive with the commercial assay. Sampietro et al. [1996] report a 73.8% agreement between their own PCR and the HGV Kit on a smaller sample. Sequencing of the RT-PCR products indicated that the assay which we developed amplified only GBV-C RNA.

The reason for the relatively high prevalence of GBV-C in blood donor populations worldwide is yet to be determined [Moaven et al., 1996; Jarvis et al., 1996; Alter, 1996]. Several mechanisms of transmission have been proposed to explain this finding, which is higher than HCV seroprevalence in most parts of the world [Jarvis et al., 1996; Alter, 1996; Stark et al., 1996]. Although the transmission of GBV-C through contaminated blood is the most well defined mode of acquisition [Masuko et al., 1996; Orito et al., 1996; De Lamballerie et al., 1996; Schmidt et al., 1996; Harrison, 1996; Stark et al., 1996], other transmission routes including vertical, sexual, and arthropod-borne are possible. Vertical transmission has been reported in both Australia [Moaven et al., 1996] and in Germany [Stark et al., 1996], although a report from Taiwan [Lin et al., 1996] indicates that mother-to-baby transmission is rare in a low-risk population. Dawson et al. [1996] have suggested that mosquitoes may aid in the spread of the virus, although no studies on insect transmission have been carried out to date. Stark et al. [1996] in Germany have noted that sexual contact may be a means of spreading the virus, with their study data indicating that sexual transmission of GBV-C does occur in the homosexual and bisexual populations which they studied. A recent report also implicates sexual transmission in heterosexuals, with five of 23 (21.7%) positive partners also becoming infected, and 13.9% of female prostitutes in the study positive for GBV-C [Rubio et al., 1996]. A Chinese study has indicated that GBV-C infection is highly prevalent in prostitutes (11.4%), possibly related to multiple sex partners [Wu et al., 1997]. However, in our study, other markers associated with multiple sexual exposures (HIV and syphilis serology), which are routinely measured in blood donors, were positive at low levels in this group, suggesting that sexual transmission is not a prominent route in our population. The basis for the high prevalence in blood donors in Gauteng remains unknown, but probably represents a combination of the above mechanisms. A significant finding in our study was the difference in GBV-C prevalence between black and white blood donors. Whilst the factors accounting for this difference are not known, there is well documented racial discordance for other hepatitis viruses in South Africa, including HBV [Schoub, 1992] and hepatitis A virus [Martin et al., 1994].

The implications of the high prevalence of GBV-C RNA in the blood donor population in this South African study are uncertain. If GBV-C is shown to play a role in disease, the findings in this study may have important implications for future screening of volunteer blood donor populations, and may affect adversely the available blood supply.

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